Massachusetts Department of Public Health

State Laboratory Institute

A Monthly Newsletter of the Bureau of Laboratory Sciences

Volume 1, Number 8 August, 1999

Bacteriology Reference Laboratory

by Ellen A. Silva

The Bacteriology Reference Laboratory (BRL) identifies unusual or difficult to identify bacteria, provides serological subtyping and confirms the identity of subcultures received from other laboratories. These services are provided to hospital and private laboratories to facilitate disease diagnosis, to health departments to assist in outbreak investigations, and to public health epidemiologists to assist in surveillance and disease investigation. Data from the BRL are important to disease surveillance programs for monitoring trends and identifying emerging diseases. These data often provide an early warning of health risks, such as the recent contamination of processed meats with *Listeria monocytogenes*.

Over time, the predominant organisms submitted for identification have changed from the Gram-negative rods, such as *Acinetobacter* spp., *Pseudomonas* spp., and *Pasteurella* spp, to the Gram-positive coccal and coryneform organisms. This shift is due, in part, to the greater recognition of the clinical significance of these and other newly described Gram-positive species, and also, to the inability of commercial rapid test systems to accurately identify these organisms.

However, Gram-negative pathogens remain significant among the organisms identified in the BRL. Among those frequently isolated and identified is *Bordetella pertussis*. An increasing incidence of pertussis among children older than eleven years of age has been found due to the loss of vaccine immunity over time. Clusters of pertussis cases at secondary schools have occurred, especially among students involved in close contact sports. The BRL provides culture kits for the isolation and identification of *Bordetella* spp. Once the specimen has been obtained and cultured, it is best to transport it by courier for same-day delivery to the laboratory. If an overnight transport service is used, the specimen should be sent with cold packs. Any culture that can not be transported immediately should be refrigerated until it can be transported.

Other Gram-negative organisms that are isolated and identified in the BRL are members of the genus *Legionella*. The predominant species found is *Legionella pnemophila* serogroup 1, the same organism that was responsible for the American Legion outbreak in Philadelphia, where the organism was first recognized in 1976. *Legionella pneumophila* other than serogroup 1, *L. bozemanni*, *L. dumoffi*, *L. longbeachae* and *L. micdadei*, as well as other species, have been sporadically isolated in specimens from patients residing in Massachusetts. Primary specimens submitted for the examination of *Legionella* must be transported on ice to prevent the overgrowth of normal flora. Transport kits for the submission of specimens for the examination of *Legionella* or *B. pertussis* may be obtained by calling the Bacteriology Office at (617) 983-6640 or by FAX request to (617) 983-6618.

Testing in the Reference Laboratory consists of conventional biochemical methods, as recommended by the Centers for Disease Control and Prevention (CDC) in Atlanta, GA. Because these sensitive testing methods are used, nearly all referred specimens are identified, with less than 5% of the isolates not identified as a known species. At the request of the sender, unidentified isolates are referred to CDC for additional testing. If CDC is also unable to identify the organism, it is reported as such and placed in their culture collection and database. Substantial numbers of submissions of similar unidentified organisms may lead to recognition of emerging pathogens. Serotyping of organisms causing reportable diseases is also an important aspect of the testing done by the BRL. Often, this information is a significant factor in the early detection of an outbreak, as exemplified by that of bacterial meningitis at a Massachusetts nursing home this past winter.

continued on page 2

Importance of Pre- and Post-Analytical Components in a QA Program

by Charles Reynolds

In my role as a program specialist for the New England Region of the Health Care Financing Administration (HCFA), I am often asked about quality assurance or continuous quality improvement issues in the clinical laboratory and how the Clinical Laboratory Improvement Amendments (CLIA) regulations apply to these issues. The CLIA regulations do not require a Continuous Quality Improvement (CQI) approach, but do require a QA system that covers the entire laboratory test system including the pre-analytical, analytical and post-analytical components. Although the CLIA regulations do not require CQI, I encourage all laboratories to develop a CQI approach when instituting their QA programs. Studies have shown that 85 to 90 percent of all test errors are associated with the pre-analytical and post-analytical components of the test system. The causes of these errors are typically preventable with a thoughtful QA program. The test system and workflow is unique for each laboratory. Therefore, a careful assessment must be made in each

continued on page 3

In This Issue

Grants, Projects & Publications

Use of Pulsed-field Gel Electrophoresis to Aid Epidemiologic Investigation of *Shigella sonnei* Outbreaks

by Amanda Goddard

Following an investigation by state epidemiologists of a shigellosis outbreak in Massachusetts, illnesses were linked to a single restaurant. Clinical specimens were submitted to SLI, and *Shigella sonnei* was isolated from twenty-three patrons and seven of sixteen employees of the restaurant. These specimens provided an opportunity to evaluate the ability of PFGE to identify linked cases in a *S. sonnei* outbreak. For PFGE to be useful in an outbreak investigation, the *S. sonnei* strains circulating in the community must vary significantly by PFGE analysis, i.e. there must be a large number of PFGE patterns that differ by a significant number of bands. Under such conditions, epidemiologic association is supported by PFGE test results when isolates are found to have indistinguishable or closely related patterns.

Shigella sonnei is not readily differentiated based on biochemical characteristics using conventional microbiological testing. However, Cameron et al. (1998) have shown that PFGE is a highly specific method of subtyping for *S. sonnei*. They investigated a prolonged outbreak of *S. sonnei* that occurred in North America from 1994-1996 in traditionally observant Jewish communities. The outbreak subtype consisted of several patterns that differed by no more than three bands. The explanation hypothesized for the finding of multiple, highly-related PFGE patterns was that genetic material was added, deleted, or rearranged over the course of the sustained outbreak or that a number of related strains were introduced independently but transmitted simultaneously. Tenover et al. (1995) reported that two to three band differences are associated with changes from a single genetic event, while a seven-band difference may indicate that three or more independent genetic events have occurred. Therefore, isolates that have PFGE test results with seven or more band differences are interpreted as not supporting an epidemiologic association.

The patrons in the Massachusetts outbreak were exposed at the restaurant over a three-day period. PFGE was performed on XbaI restricted total genomic DNA digests of thirty patron and employee isolates. Five PFGE patterns were observed that varied by no more than three bands. These patterns were considered closely related and to represent the outbreak subtypes. Even though the duration of the outbreak reported by Cameron et al. was significantly longer than the MA outbreak, the patterns associated with the outbreak also differed by no more than three bands.

For comparison, PFGE was performed on sixty *Shigella sonnei* isolates that were not associated with the restaurant outbreak. These *S. sonnei* isolates were received at SLI immediately prior to, during and up to five months after the occurrence of the outbreak. Fifty-eight of these isolates had PFGE patterns that differed from the outbreak strain by at least eleven bands, and were therefore not related to the outbreak. Two isolates differed from the outbreak strain by six bands, but they were epidemiologically unrelated. There were forty-eight unique PFGE patterns observed among the sixty isolates. These findings indicate that in Massachusetts there is considerable variability of *S. sonnei* as determined by PFGE analysis, and therefore, the method has good discriminatory power for outbreak investigations. Further studies will be done with these isolates to determine if the absence or presence of plasmids contributes to the banding differences observed among the outbreak subtypes.

References: Cameron, D. N., J. Sobel, J. Ismail, N. Strockbine, M. Williams, P. S. Diaz, B. Westley, M. Rittmann, J. DiCristina, H. Ragazzoni, R. V. Tauxe, and E. D. Mintz. A Prolonged Outbreak of *Shigella sonnei* Infections in Traditionally Observant Jewish Communities in North America Caused by a Molecularly Distinct Bacterial Subtype. J. Infect. Dis. 1998; 177: 1405-1409. Tenover, F. C., R. Arbeit, R. V. Goering, P. A Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing. J. Clin. Microbiol. 1995; 33: 2233-2239.

Note: This work was presented at the General Meeting of the American Society for Microbiology, May 30-June 3, 1999.

Bacteriology Reference Laboratory

continued from page 1

The outbreak was caused by *Neisseria meningitidis* serogroup Y. With the threat of bioterrorism in the US, the immediate submission of suspect organisms is of vital importance. Consultation regarding suspect organisms may be obtained by calling the BRL at (617) 983-6607.

Timely submissions of high quality specimens are essential to public health surveillance activities. Subcultures of isolates should be pure, actively growing (generally 18-24 hours old), and submitted in a tightened, screw capped tube on any suitable slanted carbohydrate-free medium that supports good growth. A Chocolate Agar slant is the preferred medium. A completed laboratory patient history form, including the presumptive identification of the organism, must accompany each isolate. These forms are available from the Bacteriology office by calling (617) 983-6600 or by FAX request to (617) 983-6618.

The Bacteriology Reference Laboratory also receives sera for referral to the CDC to aid in the diagnosis of certain bacterial, fungal and parasitic diseases. Serum from patients with unusual infectious diseases, or with infectious diseases caused by organisms for which expertise and/or reliable diagnostic reagents are unavailable, may be submitted to the laboratory for referral to the CDC. Further information, consultation and submission forms can be obtained by calling (617) 983-6600.

Program Reports — Lead Testing

by Julianne Nassif

The State Laboratory Institute screens pediatric whole blood specimens to identify children with elevated blood lead levels (PbB). Children 48 months of age or younger are required to be tested at least annually in accordance with Massachusetts Department of Public Health regulations. Children who are determined to be at high risk for lead poisoning, due to poor housing conditions or a family history of lead poisoning, are tested at an earlier age and more frequently. Samples, which are received from nearly 500 health care providers, are collected usually at routine well childcare visits.

PbB are measured by graphite furnace atomic absorption spectroscopy (GFAAS) with Zeeman background correction. Samples are analyzed on the day of receipt and results reported the following day to physicians and to regional state public health nurses. Private laboratories testing specimens from Massachusetts' children are required to report all blood lead data to the Department of Public Health. These data are merged with the SLI data to produce a comprehensive patient based database. In fiscal year 1998, the SLI reported results for 150,876 samples, and received 160,485 PbB test results from private laboratories. The distribution of PbB for all test results reported in FY 1998 is noted below.

PbB(μg/dL)	≤4	5-9	10-14	15-19	20-69	≥70	Total
No. of Tests	203,000	82,305	17,507	4,906	3,592	51	311,361
Percent	65.2	26.4	5.6	1.6	1.2	<0.1	100

In addition, SLI analyzes paint, soil, dust and drinking water samples to characterize sources of lead exposure in a child's immediate environment. Occasionally, samples of pottery, glassware, crayons, vitamin supplements and other miscellaneous sample types are analyzed to identify an individual child's source of lead exposure when usual sources of lead exposure are not the apparent source of lead poisoning. Lead is measured in samples by GFAAS for drinking water, flame atomic absorption spectroscopy (AAS) for dust and AAS or X-ray fluorescence spectroscopy for paint chips and soil. Lead paint inspectors and health agents submit samples as part of regulatory inspections; consumers concerned about potential sources of lead exposure may also submit samples through lead inspectors or health agents. Periodically, the laboratory provides testing of food products for lead levels to support food safety surveys of health and agriculture agencies. A summary of food and environmental testing for fiscal year 1998 is given below.

Sample Matrix	Number of Samples Tested
Drinking water	272
Soil	192
Household dust	183
Maple syrup	28
Paint	27
Miscellaneous	6
Total	708

Importance of Pre- and Post-Analytical Components in a QA Program

continued from page 1

laboratory to identify potential problem areas and to develop and implement a QA program to monitor for errors.

In larger laboratories, it is common to see a disconnection between specimen receiving and processing, testing and results reporting areas. One example is a laboratory that has a specimen receiving area that enters the patient and test information into a Laboratory Information System (LIS), and then processes or stores the specimens in a different area until testing. It is important to give appropriate training to individuals who process specimens and enter data into the LIS, especially if they do not have a laboratory background. This will help prevent errors such as misidentifying tests with similar names, and will help assure that tests are entered into the LIS according to the original test request.

Once a specimen is correctly processed and tested by the laboratory, reports need to be monitored for errors. A system that relies on transcription has a greater potential for error. It is not enough to report accurate test results. Attention must be given to the entire test report. Unclear report formats or information provided with the test report may lead to misinterpretation by the end users. Every laboratory should periodically reevaluate its report format and the report information provided with test results to assure clarity, as well as accuracy.

Problems often occur when laboratories manually enter results reported from outside reference laboratories into their own LIS without including, for example, accompanying interpretive information, reference ranges, and the original flags for abnormal results. Normal test results that are flagged as 'abnormal' on the test report, or visa versa, are serious post-analytical test errors regardless of the accuracy of the test result.

Laboratories that network or interface their LIS to another LIS or HIS (Hospital Information System) have an ongoing potential for post-analytical test errors. Depending on the type of electronic transmission, the lab receiving the test results must maintain a matching table containing the correct interpretive information and apply it to each test result. If the testing laboratory changes the test method, and the interpretive information (reference range) changes, the laboratory receiving the results must be notified and make the appropriate changes. Any breakdown in communication has the potential for creating reporting errors.

Laboratory QA programs should also monitor for systematic errors. Over time, whenever a change occurs in the laboratory testing system, it is necessary to examine what changes need to be made in the QA program. Often a major system change, such as a new LIS function, will require close monitoring to identify whether or not there are consequential short or long-term problem areas. Whenever possible, it is best to make system changes that will remove or reduce the potential for errors before they occur.

Charles Reynolds is a program specialist for the New England Region, Quality Improvement Branch, Division of Medicaid and State Operations, Health Care Financing Administration (HCFA).

Newsletter Editor: Marcia Stowell, EdM, MT(ASCP); Please contact the editor for additions and changes to the mailing list, comments and inquiries. Phone: 617-983-6283, Fax: 617-983-6210, E-mail: marcia.stowell@state.ma.us. Please visit our Web Site for the electronic version of this newsletter and other information about the State Laboratory Institute: www.state.ma.us/dph/bls.

Laboratory Training Activities

Response to Bioterrorism - Role of the Clinical Laboratory, State Laboratory Institute, Boston, MA - September 9, and Baystate Medical Center, Springfield, MA - November 1. A 1-day national conference to define the role of the clinical laboratory, with speakers from CDC (Sept 9 only), Public Health, FBI and the Medical Community. Call (617) 983-6285.

Bioterrorism - Frontline Preparedness & Response of the Clinical Laboratory, Worcester Centrum, Worcester, MA - October 27, held at the Region I ASM Annual Meeting. Call NEB-ASM at (617) 983-6371.

State Laboratory Training Coordinator, *Garry R. Greer, BS, (617) 983-6608, E-mail: garry.greer@state.ma.us.*
For a list of NLTN courses in your area sign on to the Web at http://www.cdc.gov/phppo/dls/nltn.htm.

The State Laboratory Institute Newsletter is a free monthly publication of the Bureau of Laboratory Sciences

Massachusetts Department of Public Health
Howard K. Koh, MD, MPH, Commissioner

Bureau of Laboratory Sciences
Ralph Timperi, MPH, Assistant Commissioner, Director State Laboratory Institute
(617) 983-6201, E-mail: ralph.timperi@state.ma.us

Laboratories
Foodborne and Diarrheal Diseases, STD, Reference Bacteriology, *Harvey George, PhD, Director, (617) 983-6602*
Virology/HIV, Mycobacteriology, Arboviral and Tickborne Diseases, *Barbara Werner, PhD, Director, (617) 983-6365*
Environmental Chemistry and Blood Lead Screening, *Julianne Nassif, MS, Director, (617) 983-6651*
Illicit Drug Analysis, Eastern Massachusetts, *Kevin McCarthy, BS, Director, (617) 983-6629*
Illicit Drug Analysis, Western Massachusetts, *Allan Stevenson, MS, Director, (413) 545-2606*

State Laboratory Institute
305 South Street
Boston, MA 02130-3597

Bulk Rate
US Postage
PAID
Boston, Ma
Permit No.
55970